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## Isolation of bacteria from the roots of maize

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**We use this protocol and it's working**

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## Abstract

Protocol used to isolate bacteria for the MAize Rhizosphere Synthetic community (MARSc) from the bulk soil, rhizosphere, and endosphere of maize.

- 1 Rhizotrons were filled with raw field soil with low inorganic nitrogen inputs, collected from diversified plots at the Marsden long-term cropping system experimental site, as described previously (1)
- 2 Two surface-sterilized, pre-germinated Phz51 or BGEM-0111-S/Phz51 (2) maize seedlings were planted in each rhizotron
- 3 Plants were grown in a growth chamber with a 16-h/8-h light/dark photoperiod at 24 °C until the V4/V5 developmental stage
- 4 Soil, rhizosphere (root surfaces and adherent soil), and endosphere samples were obtained as described previously (1), summarized with modifications below

#### Note

1. Bulk soil samples were aseptically collected (~1 g; >1 cm from a root) and then the root system was extracted
2. The rhizosphere was obtained by vortexing and sonicating the roots in 1 mM phosphate buffer (pH 7.2)
3. Washed roots were pulverized in a ball mill with 5 mL of phosphate buffer to collect endosphere samples

- 5 Aliquots of all samples were serial diluted in 1mM phosphate buffer and plated onto R2A or 1/10th strength TSA
- 6 Plates were incubated at 27°C until colonies appeared, then colonies were passaged until pure
- 7 Genomic DNA of each pure isolate was extracted using the Wizard gDNA Isolation Kit (Promega)
- 8 The full 16S rRNA gene (V1-V9) was Sanger sequenced for each isolate using the primers 8F and 1492R and identities confirmed via BLASTn (3)



## Protocol references

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